MISSOURI DEPARTMENT OF NATURAL RESOURCES
AIR AND LAND PROTECTION DIVISION
ENVIRONMENTAL SERVICES PROGRAM
Standard Operating Procedures

SOP #: MDNR-WQMS-104 EFFECTIVE DATE: August 12, 2003

SOP TITLE: Continuous or Long-Term Monitoring of Water Quality using a Dissolved Oxygen and Temperature Data Logger

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SUMMARY OF REVISIONS: Not applicable. This is a new SOP.

APPLICABILITY: Procedures outlined in this SOP apply to all ESP personnel who wish to conduct continuous or long-term monitoring of dissolved oxygen and temperature in the field.

DISTRIBUTION: MoDNR Intranet
ESP SOP Coordinator

RECERTIFICATION RECORD:

Date Reviewed				
Initials				

1.0 SCOPE AND APPLICABILITY

This Standard Operating Procedure provides the Environmental Services Program (ESP) field personnel with guidance on the operation and maintenance of the BioDevices Corporation Aqua 2002 dissolved oxygen (D.O.) and temperature data logger, hereafter referred to as Aqua 2002 data logger, during their field investigations.

2.0 HEALTH AND SAFETY REQUIREMENTS

2.1 Some field activities involve conducting work in and/or around raw and treated wastewater or polluted waters. Field personnel are encouraged to protect themselves by wearing the appropriate level of personnel protection equipment. Furthermore, staff will be required to participate in the department's medical monitoring program in accordance with divisional policy. In addition, employees should be familiar with the Hepatitis A prevention vaccine policy.

2.2 The sample collector shall use an appropriate level of personal protection based on the specific work being done. The minimum level of personal protection to be used is gloves and waders.

3.0 PERSONNEL QUALIFICATIONS

Field personnel shall have a working knowledge of field sample collection procedures (refer to MDNR-FSS-001 *Required/Recommended Containers, Volumes, Preservatives, Holding Times and Special Sampling Considerations*, MDNR-FSS-002 *Field Sheet and Chain-of-Custody Record*, MDNR-FSS-003 *Sample Numbering and Labeling*, MDNR-FSS-004 *Field Documentation*, and MDNR-FSS-018 *Sample Handling: Field Handling, Transportation, and Delivery to the ESP Lab*). In addition, will have at a minimum either attended the department-sponsored Inspection and Enforcement training or received training from an MDNR employee knowledgeable on proper sample collection procedures.

4.0 SAMPLING CONSIDERATIONS

4.1 As stated in MDNR-FSS-001, a D.O. sample has no holding time and must be analyzed immediately upon collection. To obtain accurate measurements, D.O. analysis must be performed on-site and in situ.

4.2 When deploying and retrieving an Aqua 2002 data logger, it is important that the field personnel record observations including: the time of day, weather conditions, water temperature, unusual stream/lake characteristics (e.g., septic conditions, algae growth, etc.), the stream segment where the field measurement was collected (e.g., riffle, pool or run) or the lake depth from where the D.O. was collected. Refer to MDNR-FSS-004 for further guidance regarding field documentation.

4.3 Dissolved oxygen and temperature measurements should not be collected from areas with turbulent flow, still water or from the stream bank, unless these conditions are representative of the stream reach or are required by the study objectives.

4.4 To reduce or prevent vandalism, the Aqua 2002 data loggers should be deployed in an inconspicuous area of the stream.

4.5 To prevent loss, the Aqua 2002 data loggers should be secured into the streambed and tethered to a secure object located on the stream bank (refer to section 9.5 for more details).

5.0 GENERAL OVERVIEW

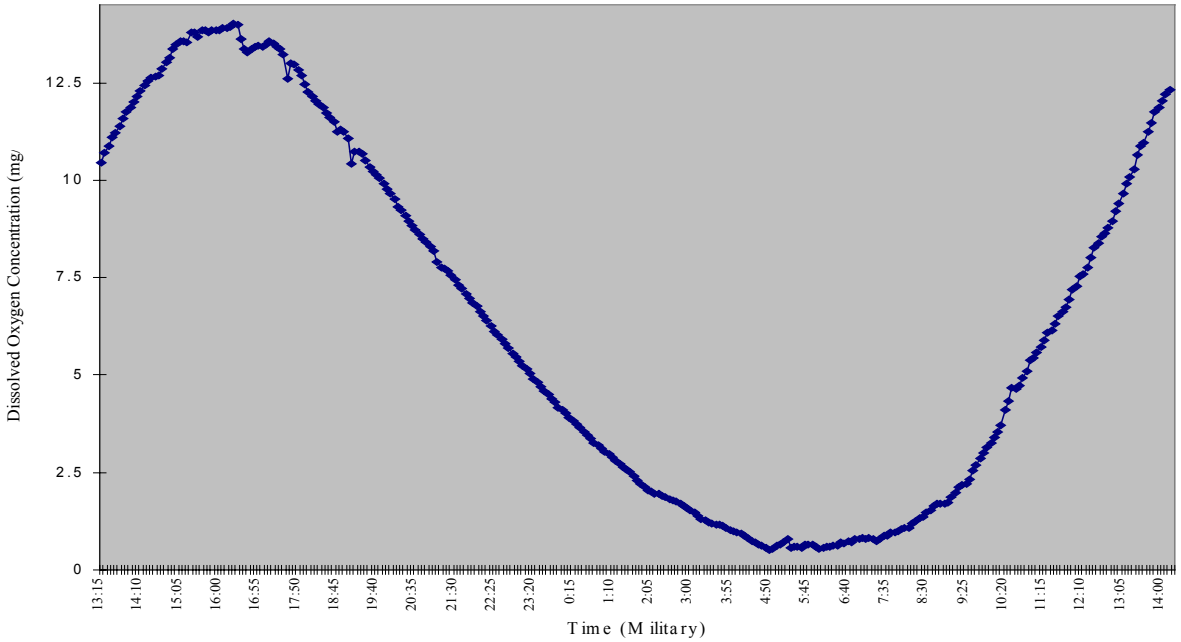
5.1 The ability of a body of water to support aquatic life is dependent on the level of D.O. contained within it. The Missouri Water Quality Standards specify that the minimum concentration of D.O. to support aquatic life is 5.0 mg/L for cool and warm waters (6.0 mg/L for cold waters).

5.2 The level of D.O. in natural water systems (e.g. streams and lakes) and wastewater depends on the physical, chemical, and biochemical activities in the body of water. One or more of these factors can influence the D.O. concentrations of a water system (refer to sections 5.2.1 – 5.2.3 for examples).

5.2.1 Oxygen becomes dissolved in water by a number of means. A water system may receive oxygen through diffusion from the atmosphere, aeration (as water tumbles through a riffle area or over a fall), and photosynthesis.

5.2.2 Non-point source runoff or discharge containing phosphorus and nitrogen compounds can significantly alter oxygen levels in water systems. These compounds are readily taken up by aquatic plants, stimulating growth and reproduction, which may result in algal blooms. As a by-product of the photosynthetic process, plants produce oxygen, initially elevating the D.O. concentration. In turn, as the plants respire, and as they die and decompose, they consume oxygen. Therefore, over a 24-hour period, the oxygen levels will generally rise through photosynthetic activity during daylight hours and then drop during the evening hours as the respiration and bacterial breakdown process occurs. Through this daily cycle, a D.O. curve can be plotted. In the presence of an algal bloom, figure 1 below is an example of what the D.O. curve may look like over a 24-hour period.

Figure 1
An example of a D.O. curve over a 24-hour period.



5.2.3 Water temperature affects the amount of D.O. that a water system can hold. Cooler water can typically hold more oxygen than warmer water can.

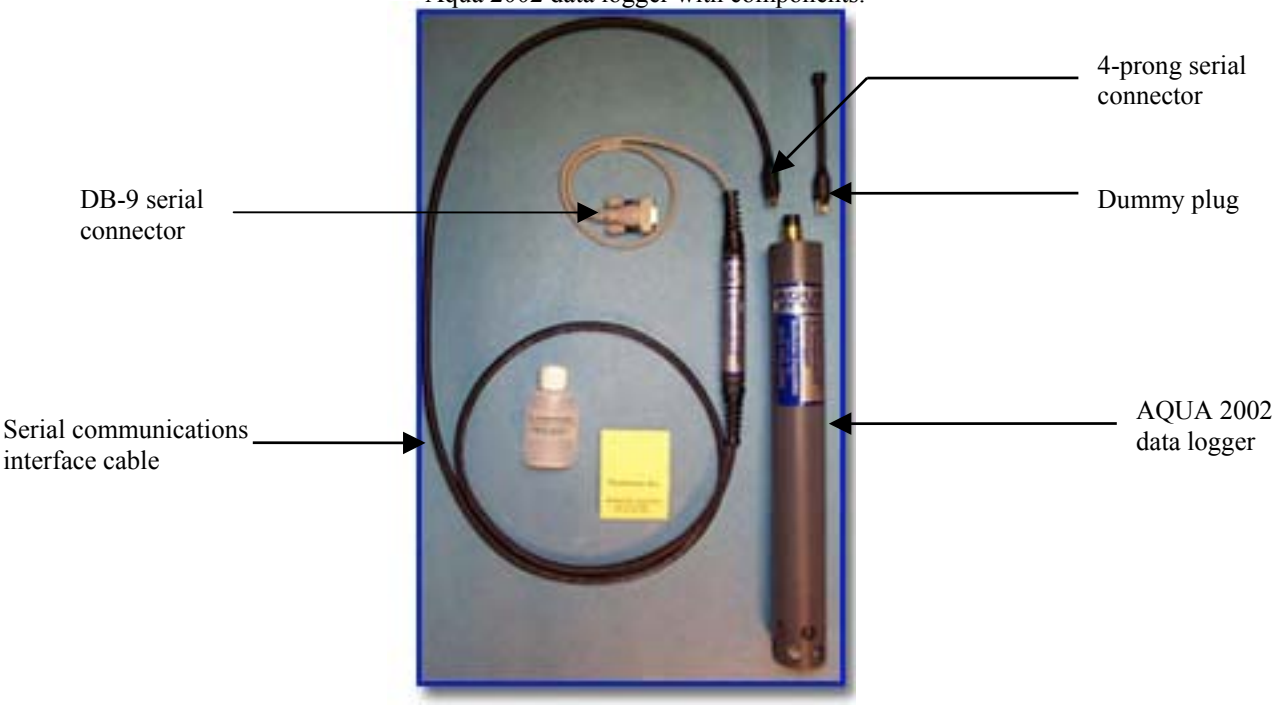
6.0 AQUA 2002 DATA LOGGER

The data loggers use the polargraphic (Clark-type) membrane probe with built-in thermistors for temperature measurement and compensation. The thin, permeable membrane stretched over the probe isolates the sensor elements from the environment, but allows oxygen and certain other gases to enter. When polarizing voltage is applied across the sensor, oxygen that has passed through the membrane reacts with the cathode, causing a current to flow. The D.O. is then expressed as mg/L or percent saturation.

7.0 COMPUTER PROCEDURES

7.1 The Aqua 2002 data logger is designed to be used with Windows 95, 98, 2000, and XP compatible PCs. The connection is made using the serial communications interface cable equipped with a standard DB-9 serial connector on one end that attaches to the serial communications port of the PC (user's choice). The opposite end of the cable is fitted with a 4-prong serial connector which mates with the connector found on top of the data logger. (Figure 2)

Figure 2
Aqua 2002 data logger with components.



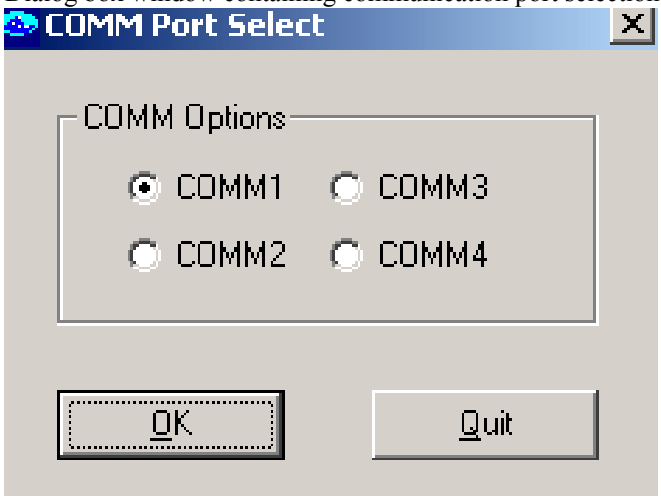
7.1.1 Turn the PC **OFF**.

7.1.2 Attach the DB-9 serial connector to the serial communications port of the PC. Then remove the dummy plug from the connector found on top of the data logger and insert the 4-prong serial connector. Turn the PC **ON**.

7.1.3 Access the BioDevices Corporation website (www.biodevicescorp.com) and download the AQUA TALK for Windows® software or select the previously installed software maintained on the user's PC.

7.1.4 A dialog box will appear prompting the user to select the appropriate serial communication port or to quit the program (Figure 3).

Figure 3
Dialog box window containing communication port selection.



7.1.5 Select the communication port on the computer and then select the **OK** box. The AQUA 2002 configuration menu will appear on the screen as shown in figure 4.

7.2 Configuration Menu

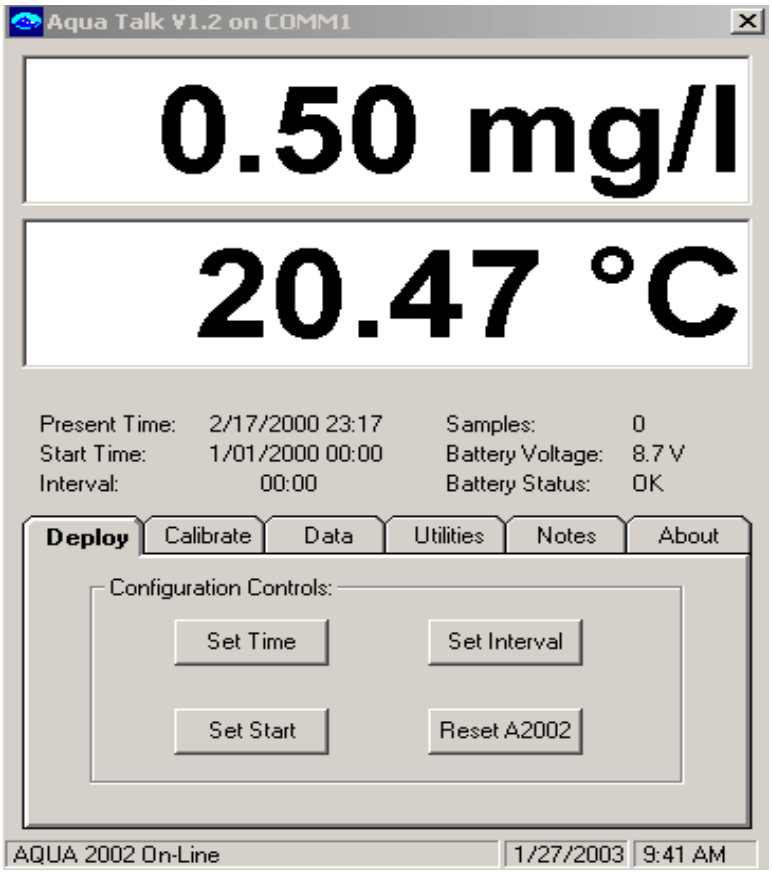
7.2.1 Shortly after the configuration menu appears, the message: **AQUA 2002 On-Line** will appear at the bottom-left corner of the menu (status line). If the wrong communication port was selected or the serial communication interface becomes disconnected from either the PC or the data logger, the message will read: **AQUA 2002 Off-Line**.

7.2.2 The AQUA 2002 configuration menu is divided into two sections (figure 4):

- Status Window Display: This window displays the current D.O. and temperature readings, present time, start time, sampling interval, number of samples logged, battery voltage, and battery status.

- Configuration Tabs: These six configuration tabs contain commands for the following functions: Deployment settings, calibration settings, data management, general utilities, notes tab, and about tab.

Figure 4
AQUA 2002 configuration menu window showing active deploy tab.



7.3 Deploy Tab

The deploy tab contains four different configuration control settings (refer to figure 4):

- Set Time: Present time, allows the user to enter the current date and time. The format for this is: two digit month and day, and a four digit year all separated by "/", followed by a space, then two digit hour and minute separated with ":" and expressed as a 24-hour clock (i.e. midnight = 0, 12 noon = 12, 1 p.m. = 13, …).

- Set Start: Start time, allows the user to set the time that the data logger will start logging D.O. and temperature data. The format for this is the same as the "Set Time" format above.

- Set Interval: Set sampling interval, allows the user to set the amount of time between logging events. The format for this is: two digit hour and minute separated by ":" and expressed as a 24-hour clock. Logging intervals can range from every minute, up to every 23 hours: 59 minutes.

- Reset A2002: Clears the memory stored in the data logger. Allows the user to clear memory with two options: clear both the data recorded and reset the time-keeping functions or clear only the data recorded.

7.4 Calibrate Tab

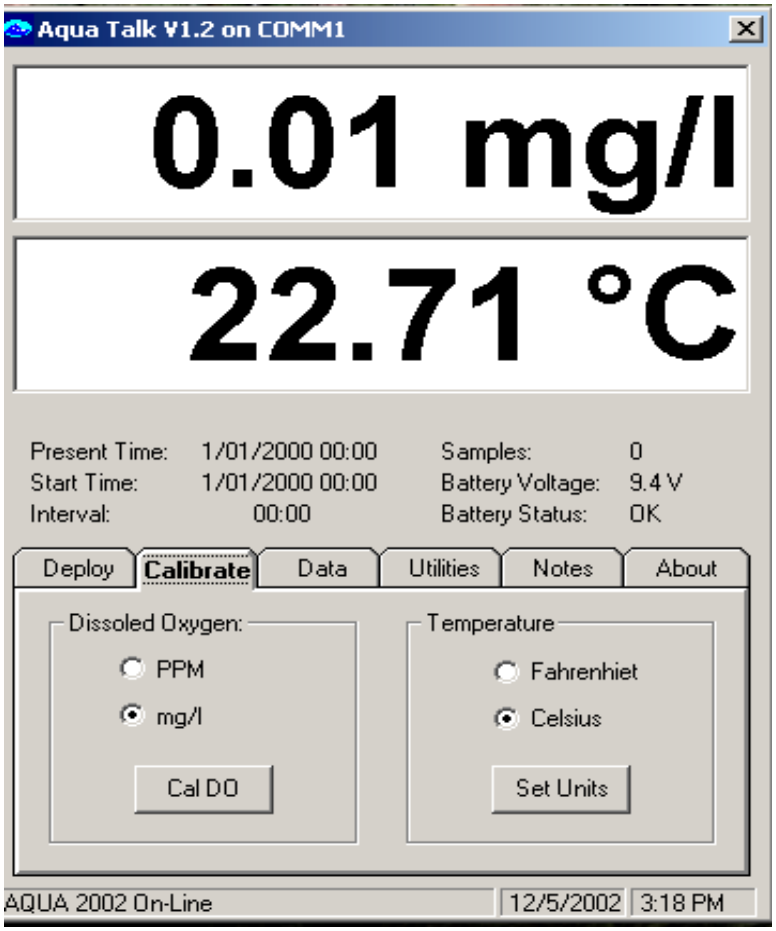
The calibrate tab contains two window settings, one for calibrating the dissolved oxygen sensor and the other for temperature unit designation (Figure 5):

- Dissolved Oxygen: This window allows the user to designate how the D.O. will be expressed; either parts per million (ppm) or milligrams per liter (mg/L). Then prompts the user to select the **Cal DO** key, which initiates the calibration of the D.O. sensor.

- Temperature: This window is used to designate how the temperature will be expressed; either Fahrenheit or Celsius. The user is then prompted to confirm the selection by selecting the **Set Units** key.

Refer to section 8.0 of this SOP for calibration procedures.

Figure 5
AQUA 2002 configuration menu window showing active calibrate tab.



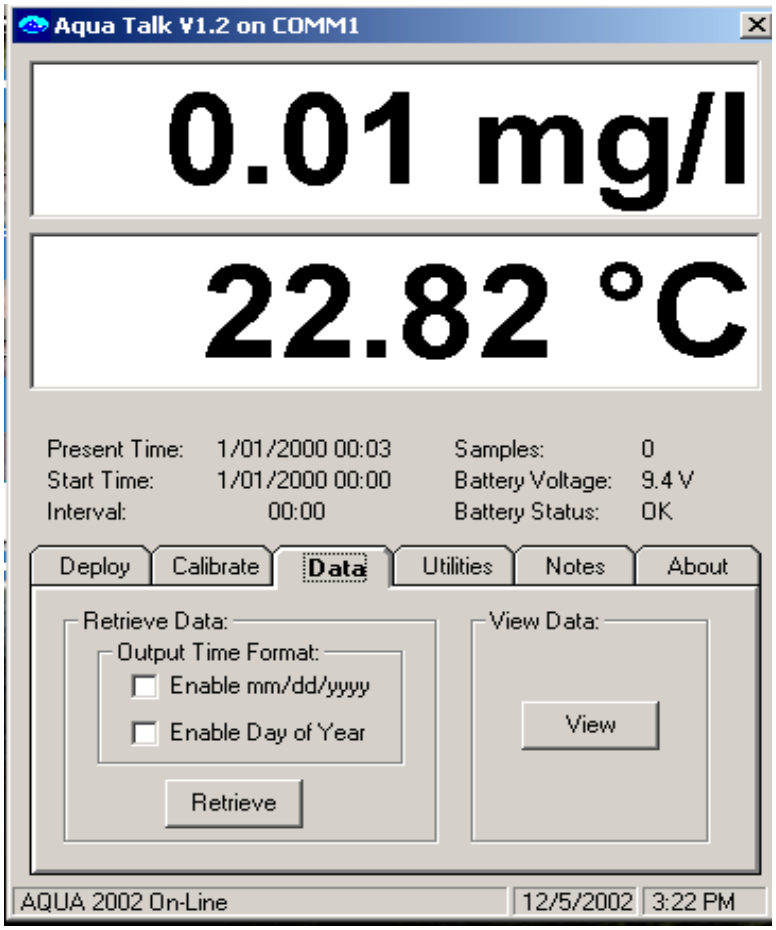
7.5 Data Tab

The data tab contains two window settings, one for retrieving data consisting of two different output time formats and one for viewing data (Figure 6).

- Output Time Format, mm/dd/yyyy: Format displays the date and the time data was logged.

- Output Time Format, Day of Year: Format displays the day of year and the fraction of day that data was logged. The format for this is: three-digit day of year, and a five-digit fraction of day separated by ".". The day of the year format does not contain information regarding the calendar year (Day 1-January 1st to Day 365-Dec 31st). Therefore, when using a spreadsheet program, the program will automatically set the year to 1900.

Figure 6
AQUA 2002 configuration menu window showing active data tab.

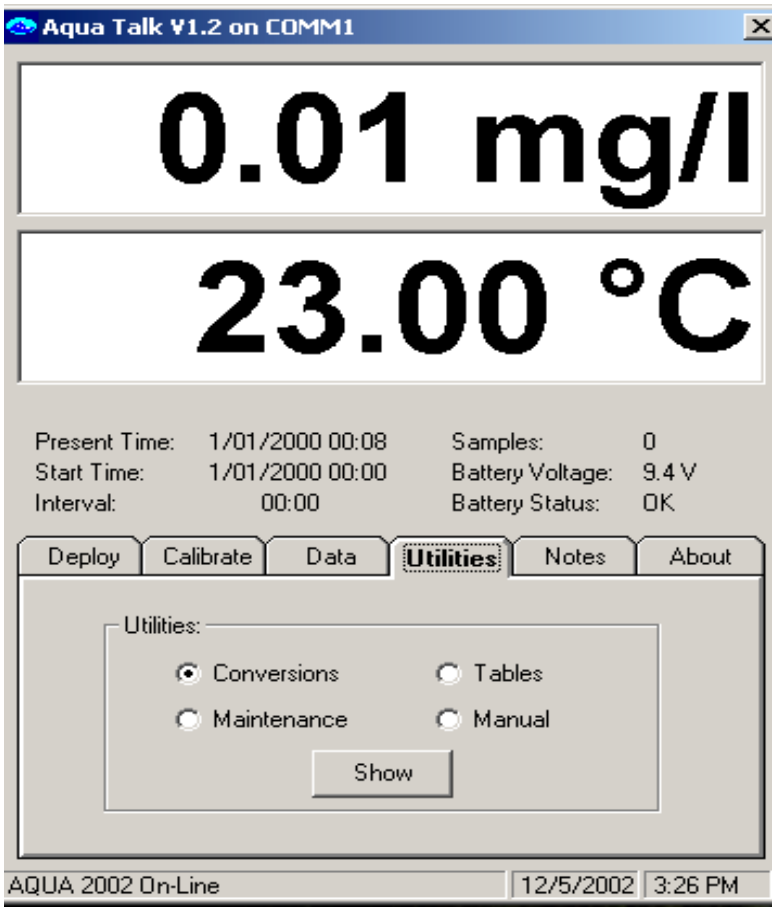


7.6 Utilities Tab

The utilities tab of the AQUA 2002 configuration menu contains four utility setups to view (Figure 7):

- Conversions
- Maintenance
- Tables
- Manual

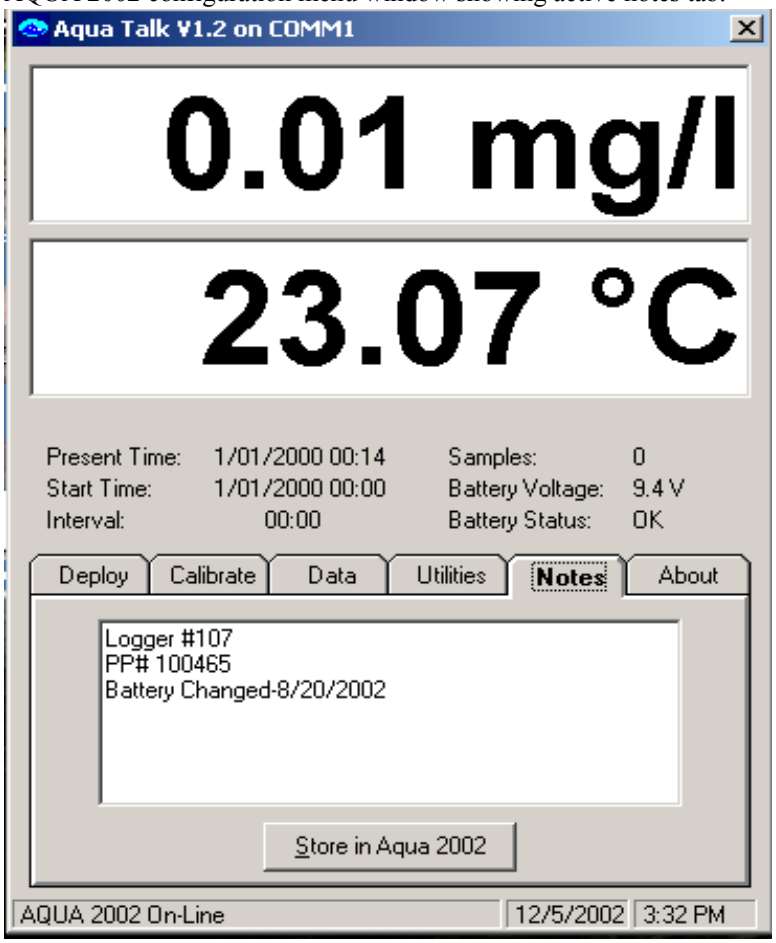
Figure 7
AQUA 2002 configuration menu window showing active utilities tab.



7.7 Notes Tab

The notes tab provides a text box where the user can record information, notes, comments, etc. regarding the instrument. The information is stored in the data logger's memory and can be updated, changed, or deleted by the user at anytime. (Figure 8)

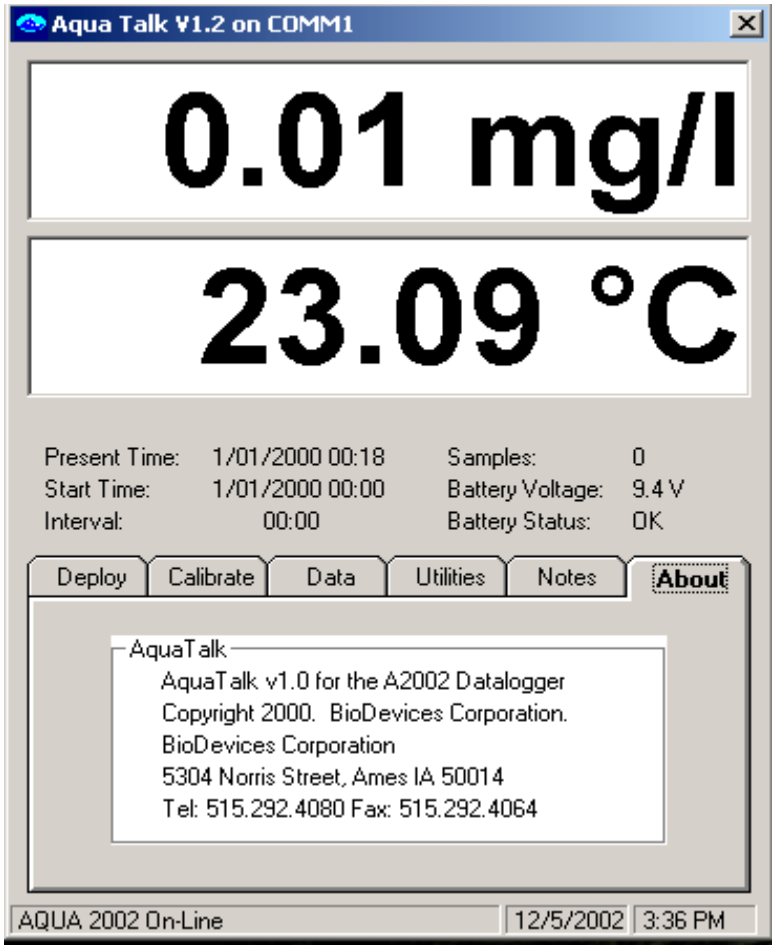
Figure 8
AQUA 2002 configuration menu window showing active notes tab.



7.8 About Tab

This tab contains the identification text box screen that displays software copyright information and BioDevices Corporation contacts information (Figure 9).

Figure 9
AQUA 2002 configuration menu window showing active about tab.



8.0 CALIBRATION PROCEDURE

8.1 Predeployment calibration is required; however, the D.O. sensor must be calibrated each time the membrane material and filling solution are changed. Furthermore, the calibration can be disturbed by physical shock, touching the membrane, fouling of the membrane, drying out of the electrolyte, or the sensor response may simply drift over time. Therefore, a periodic inspection of the probe and membrane and re-calibration is recommended. Refer to Appendix A for instructions on installing new membranes.

8.2 The operator has the choice of two calibration methods: Air-saturated water, and water-saturated air. Experience has shown that air-saturated water calibration is quite reliable and far simpler than water-saturated air when multiple unit predeployment calibration

operations are being conducted. The water-saturated air calibration method is excellent for calibrating in the field environment. Sections 8.4 and 8.5 will explain the calibration procedures by air-saturated water and the water-saturated air method, respectively.

8.3 Both temperature and pressure affect oxygen solubility; therefore, the AQUA 2002 Configuration Program compensates for these two parameters automatically during calibration. Since the AQUA 2002 constantly monitors temperature, all the user needs to provide is the "true" atmospheric pressure, which can be read on a barometer. If the "true" atmospheric pressure is not available, a rough calibration may be obtained using the local elevation. The altitudes across the state of Missouri vary from 265ft. to 1,316ft. Correction values for these altitudes range from 99% to 96%, respectively. For consistency, field personnel shall calibrate all Aqua 2002 data loggers using a 700ft. correction value (97%). With this in mind, there may be slight differences (approximately1% - 2% error) in D.O. readings collected from areas of Missouri located above or below 700ft. This corresponds to approximately a 0.3mg/L difference in D.O. ranges normally encountered in the field.

8.4 Calibration by air-saturated water using the AQUA 2002 D.O. and temperature datalogger.

8.4.1 Submerge all dataloggers (probe end) in a bucket or other container of air-saturated water and allow to stabilize for at least 30 minutes prior to calibration. The water in the container should be held at room temperature and completely air saturated. This is accomplished by filling a plastic bucket or other type of container with water, placing on a lab bench and vigorously bubbling the water with an aquarium type aerator for at least 60 minutes.

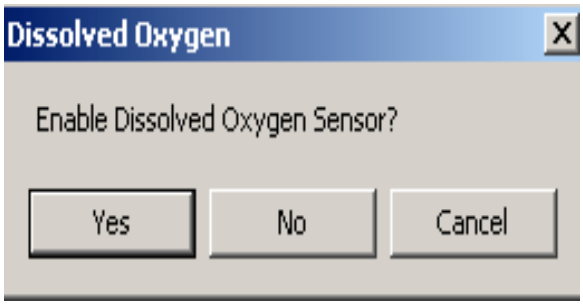
8.4.2 Attach the DB-9 serial connector to the serial communications port of the PC that is running the AQUA TALK for Windows software. Then remove the dummy plug from the connector found on top of the data logger and insert the 3-prong serial connector (refer to Section 7.1 for additional information).

8.4.3 Shortly after the configuration menu appears on the screen, select the calibrate tab of the configuration menu tabs (refer to section 7.4).

8.4.4 Once the calibrate tab is displayed, select the designated units of display for the D.O. and temperature and then select the **CAL DO** box (refer to section 7.4).

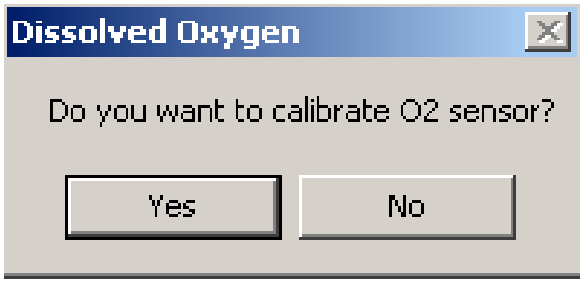
8.4.5 A dialog box will appear prompting the user to enable the D.O. sensor, with the choice of yes, no, or cancel (Figure 10). Select the **Yes** box.

Figure 10
Dialog box window containing dissolved oxygen sensor enablement selection.



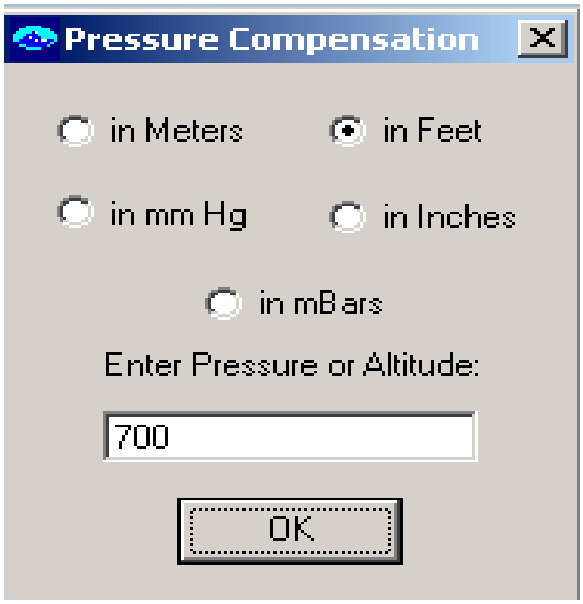
8.4.6 Once the dissolved oxygen sensor is enabled, a dialog box will appear with the message: "Do you want to calibrate O_2 sensor?" (Figure 11). Select the **Yes** box.

Figure 11
Dialog box window containing O_2 sensor calibration selection.



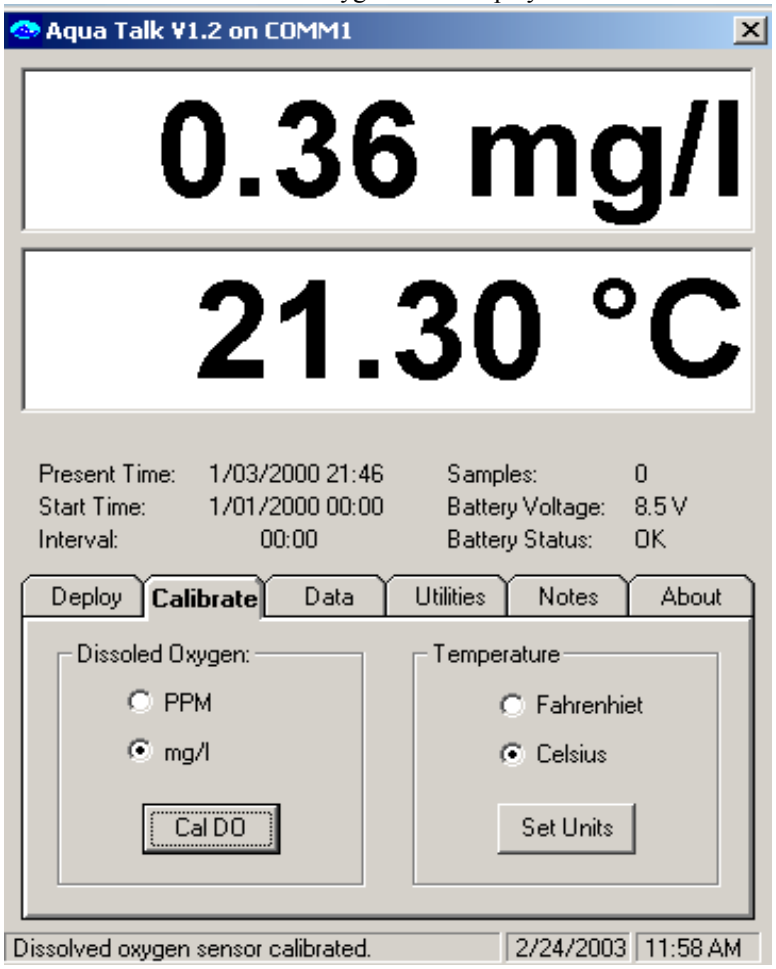
8.4.7 Once the O_2 sensor calibration is enabled, a dialog box for pressure compensation will appear. This window contains two settings, one for unit designation and the other for corresponding pressure or altitude. Then it requires the user to confirm the selection by selecting the **OK** box (Figure 12). Select **in Feet** for the unit designation, and then enter the number **700** in the required pressure or altitude box. Finally, select the **OK** box.

Figure 12
Dialog box window for pressure compensation.



8.4.8 Shortly after the pressure compensation correction has been made, the message: "Dissolved oxygen sensor calibrated" will appear at the bottom-left corner of the menu and the new calibrated dissolved oxygen value will be displayed in the status window (Figure 13). The dissolved oxygen sensor is now calibrated. To verify correct calibration, the calibration value obtained from the data logger should be compared to the value given on an oxygen solubility table or by Winkler titration. Refer to Appendix B and C for oxygen solubility table and Winkler titration methods, respectively.

Figure 13
AQUA 2002 configuration menu window status line displaying message "Dissolved oxygen sensor calibrated" (bottom-left corner of the menu), and the new calibrated dissolved oxygen value displayed in the status window.



8.4.9 Unplug the serial communications interface 3-prong plug and replace with the dummy plug. Repeat steps 8.4.1 – 8.4.8 for each data logger to be deployed. Data logger's readings should be checked against each other and against a Model 58 YSI field dissolved oxygen meter whose accuracy has been verified through the monthly field meter QA/QC checks.

8.5 Calibration by water-saturated air using the Aqua 2002 data logger.

8.5.1 The water-saturated air method is excellent for calibrating the Aqua 2002 data logger in the field. Place cap with moistened sponge over Aqua 2002 data logger probe and allow to stabilize for at least 30 minutes prior to calibration.

8.5.2 Prepare the Aqua 2002 data loggers as described in sections 8.4.2 – 8.4.8.

8.5.3 All AQUA 2002 D.O. and temperature data loggers should be checked against each other and against a Model 58 YSI field dissolved oxygen meter whose accuracy has been verified through the monthly field meter QA/QC checks.

9.0 DEPLOYMENT PROCEDURE

9.1 The day prior or morning of the deployment, calibration of each data logger by air-saturated water or water-saturated air should be performed (refer to section 8.0 for calibration procedures). Also, at this time the PC that is running the AQUA TALK for Windows software should determine the official time for synchronization of all data loggers and of watches used by field personnel who are deploying the data loggers.

9.2 After all of the verification checks have been performed (calibration and time synchronization), the "starting time" and "sample interval" should be set on each data logger, according to the guidelines given in section 7.3.

When applicable, on-site verification should be performed. Make sure that the starting time for data collection has been reached and the data loggers are collecting data.

9.3 Calibrate a YSI dissolved oxygen Model 58 field meter and then place all of the data loggers and the field meter probe into a body of water (stream, lake, etc.) near the study site, and allow a minimum of 5 minutes for acclimatization. Reminder, make sure that the dummy plug is replaced prior to submerging in water.

When acclimated, begin collecting dissolved oxygen and temperature data with the field meter, during an interval previously specified for the data loggers (e.g. 1 min.). Record field measurements along with the official time the measurements are collected, using a synchronized watch. Collect data for approximately a 5-minute period or 2-3 data logging sets. This will allow for comparisons of all data loggers to be made with the field meter following the downloading of data from each data logger (refer to section 10.0 for information regarding data retrieval).

Deploy each data logger at its designated site and, using the field meter, collect temperature and dissolved oxygen measurements at each deployment site.

9.4 It is the user's responsibility to adequately secure the Aqua 2002 data logger at the site under investigation. As stated previously, data loggers should be deployed in an inconspicuous area of the investigation site to reduce or prevent the chance of vandalism.

9.5 To prevent loss, the data loggers should be secured into the streambed and tethered to a secure object located on the stream bank. This can be achieved by clamping the data logger and its protective 2 in. Polyvinyl Chloride (PVC) sleeve to a piece of rebar or T-post driven into the streambed and tethered by rope or cable that is secured to the bank (refer to figure 14 and 15 for data logger deployment illustrations).

Figure 14
Data logger deployment setup.

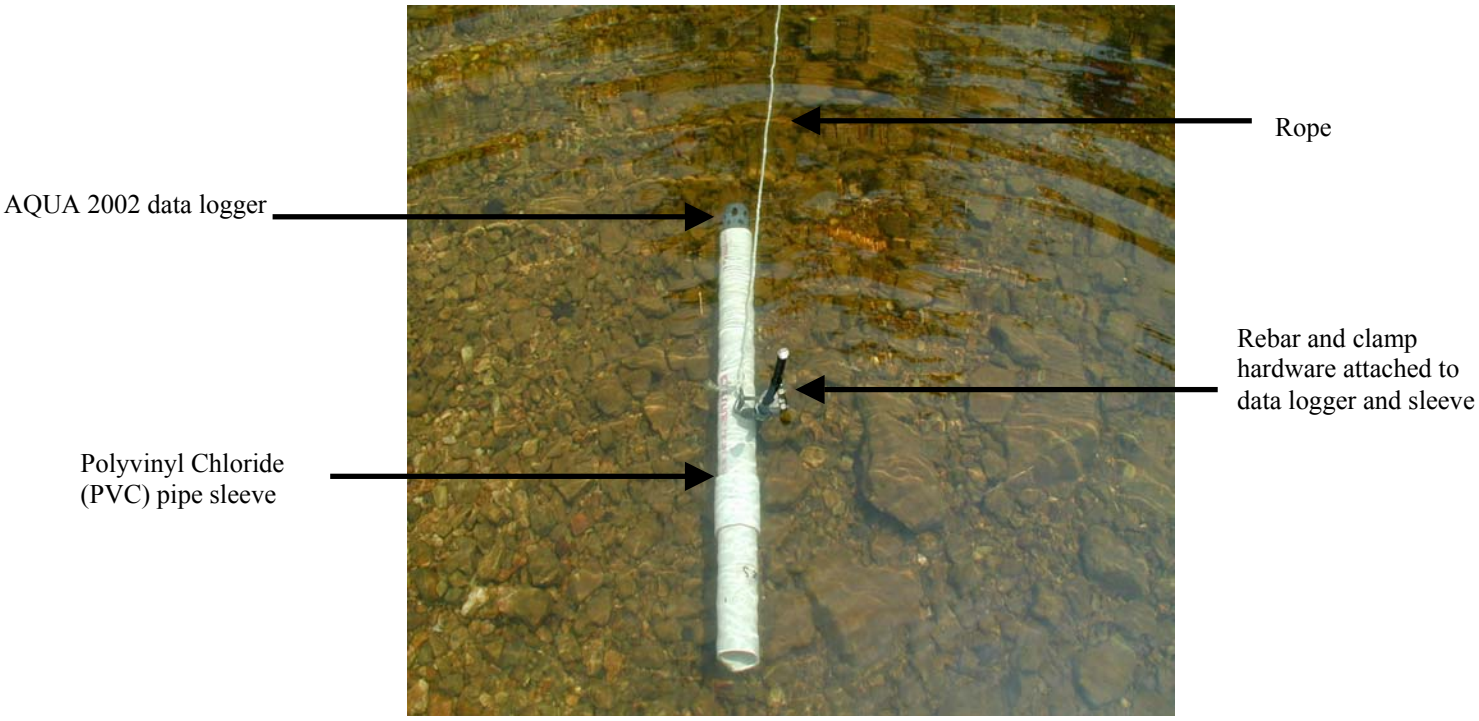


Figure 15
Data logger deployed in stream tethered to a tree on the bank.

9.6 For accurate data, it is imperative that the sensor end of the data logger remains clear of debris. The Aqua 2002 data logger has the capability of taking detailed and accurate measurements over a wide range of flow conditions, with the use of a novel membrane material that dramatically reduces flow sensitivity while maintaining rapid response. To prevent oxygen deficiency from occurring at the membrane surface, it is recommended that the sensor end of the data logger face into or perpendicular the current.

10.0 DATA VIEWING/RETRIEVAL

10.1 Data retrieval is the process of downloading the data logger data and placing the data file on a local computer for analysis. Data retrieval and viewing requires that the Aqua 2002 data logger be connected to a Windows 95, 98, 2000, or XP compatible PC that is running the AQUA TALK for Windows software (see sections 7.1 and 7.2).

10.2 Select the Data Tab on the configuration menu. This tab contains two window settings one for retrieving data and one for viewing data (Figure 6).

10.3 Logged data can be viewed by selecting the “view” button. At the top of the window is a table that shows the logged readings. Scroll up and down to view all the readings. To download the data, select the “resume” button.

10.4 In the "Output Time Format" box, select the designated boxes labeled "Enable mm/dd/yyyy" and "Enable Day of Year". Press the "retrieve" button to download the data.

10.5 On the browser window, designate a place to save the data and specify a name for the file. Save the file as a comma separated value file by adding the ".csv" extension to the end of the file name. This saves the file as a text file that can be easily read by a variety of programs such as Microsoft Excel. Then select the save button.

Note: There is a folder on the P drive called "p:\wqm\Aqua2002_Datalogger", specifically for storage of data logger data.

10.6 Once all the data has been stored as a file on the PC, a message " Loading Data…100% complete" will appear at the bottom-left corner of the menu (status line).

11.0 DATA LOGGER STORAGE

11.1 The Aqua 2002 data loggers are sophisticated electronic equipment that require care during handling and operation. The data loggers should be protected from jostling and/or sudden impacts during transportation.

11.2 For short-term storage (several days to several weeks between use), the data logger oxygen sensor must be kept moist to prevent the need for membrane replacement. This is easily accomplished by placing the oxygen sensor end of the data logger into a plastic bag or container with a water-moistened sponge or damp cloth. Be sure that the plastic bag or container provides a closed environment to prevent evaporation (resulting in cooler air temperatures) from occurring. For short-term storage, battery removal is not required.

11.3 For long-term storage (months), the manufacturer recommends storing away from extreme temperatures, with its membrane removed and filling solution drained. For long-term storage, the battery should be removed.

12.0 REFERENCES

MDNR-FSS-001 Required/Recommended Containers, Volumes, Preservatives, Holding Times and Special Sampling Considerations

MDNR-FSS-002 Field Sheet and Chain-of-Custody Record

MDNR-FSS-003 Sample Numbering and Labeling

MDNR-FSS-004 Field Documentation

MDNR-FSS-018 Sample Handling: Field Handling, Transportation, and Delivery to the ESP Lab

MDNR-WQMS-103 Sample Collection and Field Analysis for Dissolved Oxygen Using a Membrane Electrode Meter

BioDevices Corporation. 2001. Software. <http://www.biodevicescorp.com> Accessed 2003 March 25

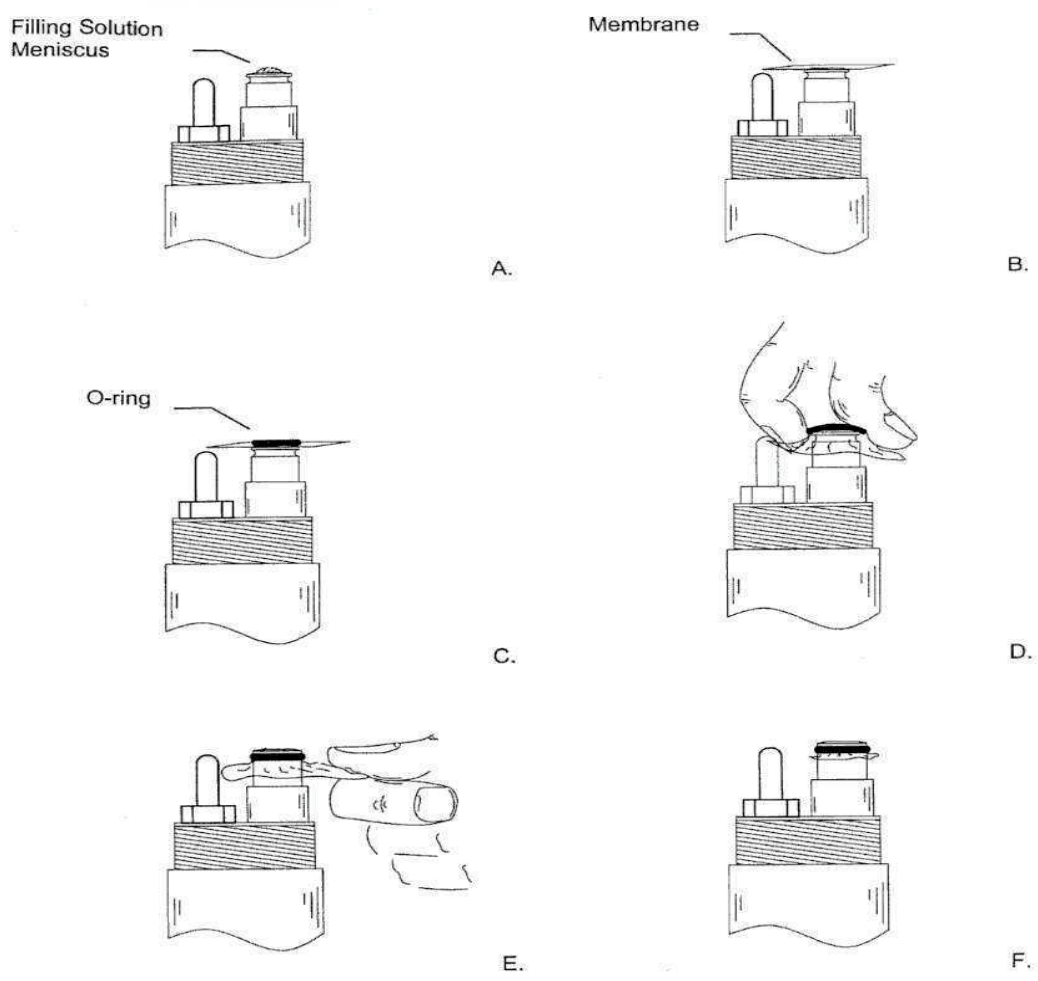
Standard Methods for the Examination of Water and Wastewater, 1998, 20th Edition, Section 4500-O B

Code of State Regulations, Title 10, Department of Natural Resources, Division 20 – Clean Water Commission, Chapter 7, Water Quality

APPENDIX A
MDNR-WQMS-104

DISSOLVED OXYGEN SENSOR

MEMBRANE INSTALLATION



Step 1. Unscrew the sensor guard by turning it counterclockwise.

Step 2. Remove the small O-ring and membrane.

Step 3. Fill the sensor cavity with new filling solution (potassium chloride) until a convex meniscus protrudes above the sensor cavity. If any bubbles appear in the cavity, they must be removed. (Diagram A)

Step 4. Center and gently place the membrane on the filled sensor cavity so that it balances by itself. (Diagram B) Make certain no air bubbles are trapped under the membrane. If any air bubbles appear repeat steps 3-4.

Step 5. Next, center and place the O-ring on top of the membrane. (Diagram C)

Step 6. While applying equal pressure with your thumb and index fingers to the opposite sides of the O-ring, smoothly push down and roll the O-ring into position. (Diagram D) Furthermore, if any air bubbles appear repeat steps 2-6.

Step 7. Inspect the membrane for wrinkles. Wrinkles can generally be removed by lightly tugging on the excess membrane around the wrinkle. (Diagram E) If any wrinkles cannot be eliminated, repeat steps 2-7.

Step 8. Trim off the excess membrane beneath the O-ring. (Diagram F) Wipe off any excess filling solution and reinstall the sensor guard.

CARE AND MAINTENANCE

- Once a new membrane is installed, submerge the data logger sensor in a container of water overnight before calibrating to ensure that the membrane has fully relaxed.

- Once the data logger sensor has been installed with filling solution and membrane, it is imperative that the sensor be kept in a container of water or cap with a water-moistened sponge. This will prevent the sensor from drying out and forming bubbles in the filling solution. The probe can also be wrapped loosely with a damp cloth or paper towel taking care not to touch the membrane. If wrapped in a damp cloth or towel, the probe should be placed in a closed environment (e.g. plastic bag or container) to prevent evaporation.

- If bubbles form under the membrane or the membrane becomes damaged, the filling solution and membrane should be replaced to prevent logging of inaccurate data.

- If the membrane becomes fouled with algae or other debris, clean by gently wiping across the membrane with a soft tissue.

APPENDIX B
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OXYGEN SOLUBILITY TABLES

TABLE I. SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER SATURATED AIR AT 760 mm Hg PRESSURE

Temp °C	Solubility mg/L	Temp °C	Solubility mg/L	Temp °C	Solubility mg/L
0	14.62	16	9.87	32	7.31
1	14.22	17	9.67	33	7.18
2	13.83	18	9.47	34	7.07
3	13.46	19	9.28	35	6.95
4	13.11	20	9.09	36	6.84
5	12.77	21	8.92	37	6.73
6	12.45	22	8.74	38	6.62
7	12.14	23	8.58	39	6.52
8	11.84	24	8.42	40	6.41
9	11.56	25	8.26	41	6.31
10	11.29	26	8.11	42	6.21
11	11.03	27	7.97	43	6.12
12	10.78	28	7.83	44	6.02
13	10.54	29	7.69	45	5.93
14	10.31	30	7.56	46	5.84
15	10.08	31	7.43	47	5.74

TABLE II (in part). CALIBRATION VALUES FOR VARIOUS ATMOSPHERIC PRESSURES AND ALTITUDES.

Pressure inches Hg	mm Hg	kPa	Altitude Ft.	m	Calibration value (%)
30.23	768	102.3	-276	-84	101
29.92	760	101.3	0	0	100
29.61	752	100.3	278	85	99
29.33	745	99.3	558	170	98
29.02	737	98.3	841	256	97
28.74	730	97.3	1126	343	96
28.43	722	96.3	1413	431	95
28.11	714	95.2	1703	519	94
27.83	707	94.2	1995	608	93
27.52	699	93.2	2290	698	92
27.24	692	92.2	2587	789	91
26.93	684	91.2	2887	880	90
26.61	676	90.2	3190	972	89
26.34	669	89.2	3496	1066	88
26.02	661	88.2	3804	1160	87
25.75	654	87.1	4115	1254	86
25.43	646	86.1	4430	1350	85

APPENDIX C
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WINKLER TITRATION METHOD

Equipment required:	**Reagents required:**
• 4-300 mL BOD bottles, glass stoppered • 25 mL titration buret with support stand • 250 mL graduated cylinder • eye-dropper • white background sheet	• manganese sulfate solution • alkaline iodide-azide solution • sulfuric acid • starch indicator solution • deionized water (dilution water) • sodium thiosulfate solution

Procedure:

1. With the D.O. instrument OFF, check the mechanical zero, if applicable.
2. Fill the four BOD bottles with dilution water.
3. Place the probe in one of the bottles. If applicable, turn the meter selection knob to RED LINE. Allow the meter to warm up 20-30 minutes for the probe to polarize.
4. To the remaining three bottles of dilution water:
   a) Add two droppers of manganese sulfate solution below the water line.
   b) Shake the alkaline iodide-azide solution and add two droppers above the water line.
   c) Stopper the bottles and invert bottle 15 times **(Do not allow air bubbles to be trapped in the bottles)**. A brown flocculent indicates the presence of D.O.
   d) Let the floc settle half way down the bottle.
   e) Invert the bottles another 15 times.
   f) Let the floc settle a third of the way down the bottle.
   g) Add two droppers of concentrated H_2SO_4.
   h) Stopper the bottles and invert 5 times **(Do not allow air bubbles to be trapped in the bottles)**.
   i) Pour off 97-98 mLs (to make room for the titrant).
   j) Rinse the buret with sodium thiosulfate solution twice, then fill and zero.
   k) Add titrant to the BOD bottles until a pale straw-yellow color develops (approximately 7 mLs); without shaking the starch solution, draw one dropper full from the clear layer, add to each bottle and swirl without aerating the sample.
   l) Add titrant one drop at a time until the sample just turns clear (a white background behind the bottle will help determine when endpoint is reached).
   m) Average the values for three bottles. This is the mg/L value of dissolved oxygen for the dilution water.